

CUT&Tag: S2 cells were collected by centrifuging at 300 g for 5 minutes and washed with 1x PBS. Nuclei were extracted by incubating with NE1 buffer (10 mM HEPES pH7.9, 10 mM KCl, 0.1% Triton X-100, 20% glycerol, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail) for 10 minutes on ice. The nuclei were then centrifuged at 500 g for 8 minutes and resuspended in Wash Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail). Afterwards, 100,000 S2 nuclei were bound to 5 μ L of ConA beads (Bangs Laboratories) for 10 minutes at room temperature. Prior to binding, ConA beads were activated via 2 washes with Binding Buffer (10 mM HEPES pH7.9, 10 mM KCl, 1 mM CaCl_2 , 1 mM MnCl_2). Subsequently, nuclei were incubated with primary antibody diluted 1:100 in Antibody Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail, 2mM EDTA, 0.1% BSA) at 4 °C overnight. Afterwards, nuclei were incubated with secondary antibody diluted 1:100 in Wash Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail) for 45 minutes at RT. Excess antibodies were removed via x2 washes with Wash Buffer. Nuclei were then incubated with pA-Tn5 diluted 1:200 in 300 Wash Buffer (20 mM HEPES pH7.5, 300 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail) for 1 hour at RT. Excess pA-Tn5 was removed via 3x washes with 300 Wash Buffer. Nuclei were washed thrice to remove excess pA-Tn5 and tagmentation buffer (20 mM HEPES pH7.5, 300 mM NaCl, 10 mM MgCl_2 , 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail) was added to perform tagmentation for 1 hour at 37 °C. The reaction was then stopped by doing a wash with 10 mM TAPS and stripping off pA-Tn5 by resuspending nuclei in 0.1% SDS buffer (10 mM TAPS with 0.1% SDS) and incubating for 1 hour at 58 °C. Sequencing libraries were then amplified. The nuclei suspension was mixed with 15 μ L of 0.67% Triton X-100, 2 μ L of 10 mM i7 primer, 2 μ L of 10 mM i5 primer and 25 μ L of 2x NEBNext Master Mix (NEB). The following PCR conditions were used: 1) 58 °C for 5 minutes, 2) 72 °C for 5 minutes, 3) 98 °C for 30 seconds, 4) 98 °C for 10 seconds, 5) 60 °C for 15 seconds, 6) Repeat steps 4-5 11 times, 7) 72 °C for 2 minutes, 8) Hold at 4 °C. Sequencing libraries were then purified using 1.3x HighPrep PCR Cleanup System (MagBio) beads as per manufacturer's instructions. Sequencing was performed using paired-end 25bp sequencing reads.